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Biopharmaceutical Study of the Hepato-biliary Transport of Drugs. I. Hepato-biliary Transport of Non-metabolizing Organic Anionic Compounds in Rat

KANJI TAKADA, YOUKO MIZOBUCHI, and SHOZO MURANISHI

Faculty of Pharmaceutical Sciences, Kyoto University[1]

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Bromphenol blue (BPB) and *p*-acetylaminohippuric acid (PAAH) were studied with rats to understand the basic principles of hepato-biliary transport process. At higher doses of BPB, the hepato-biliary transport capacity appeared to be saturated. At 30 min after the intravenous administration of BPB (5 μmole/300 g body weight), bile/plasma concentration ratio (B/P ratio) was 894±99, liver/plasma concentration ratio (L/P ratio) was 22±5 and bile/liver concentration ratio (B/L ratio) was 42±8. In the case of PAAH (6 μmole/300 g), B/P ratio was 43±15, L/P ratio was 0.47±0.27 and B/L ratio was 12±30. Sulfobromophthalein (BSP) inhibited the L/P and B/L ratios of BPB and BPB inhibited the L/P and B/L ratios of PAAH. With rat liver cell suspensions, BPB was entrapped very rapidly and its cell/medium concentration ratio was about 21 at 30 μg/ml, which was depressed by BSP. With equilibrium dialysis, 95.6±1.5% of BPB and 11.3±3.4% of PAAH were bound to the liver homogenate. These results suggest that these organic anions are transported from blood into bile at least two processes, namely hepatic uptake and biliary excretion, and that the hepatic uptake of these compounds is mainly due to the binding to the substances which exist in the liver cells.

Although up to this time many studies have been done about the biliary excretion of sulfobromophthalein (BSP),[2-5] it is thought that BSP is not suitable for a model compound to understand the basic principles of the hepato-biliary transport process because of being complicated by metabolic reactions before its appearance into bile. For this reason, instead of BSP, its metabolite, phenol 3,6-dibromophthalein disulfonate was employed by Whelan, *et al.*[6]

Bromphenol blue (BPB)[7] is excreted into rat bile as an unchanged form and *p*-acetylaminohippuric acid (PAAH), conjugated form of *p*-aminohippuric acid,[8,9] appears also unchangedly into rat bile. Thus in this study, BPB and PAAH were chosen as metabolic stable model compounds among the organic anions that are actively secreted into bile. It is considered that the mechanisms involve at least three steps, in the hepatic excretion of these compounds as follows: (1) uptake by the liver parenchymal cells, (2) intracellular transport, (3) secretion from the liver parenchymal cells into bile canaliculi.[10] In the present study of the hepatic uptake and the biliary excretion of BPB and PAAH *in vivo* or *in vitro* in rat, the characteristics of the transfer processes are examined and elucidation of the mechanism by which these compounds are transported mainly by liver cells uptake and by bile canaliculi secretion is tried.

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Experimental

Materials——*p*-Acetylaminohippuric acid was synthesized by acetylation of *p*-aminohippuric acid in our laboratory. Bromphenol blue and *p*-aminohippuric acid were purchased from Nakarai Chemicals Co., Ltd. Sulfobromophthalein sodium was supplied by Tanabe Pharmaceutical Co., Ltd. as "BSP injection 'Eiken'."

Operational Methods——Male Wistar rats, 270—300 g, were anesthetized by intraperitoneal injection of sodium pentobarbital. The renal pedicles were ligated and the common bile duct was cannulated with a polyethylene tube (0.75 mm in diameter). Body temperature was monitored *via* rectal probe and was maintained at 37±1° by a warming lamp over the animal during the experimental period. Compounds were administered into a femoral vein by a single injection in a 30 sec period. BPB and PAAH were dissolved in isotonic phosphate buffer solution (pH 7.4) prior to injection. Bile samples were collected for three 10 min periods into tared bottles and volume was considered to be equivalent to the weight of the bile. At 30 min after the administration of BPB or PAAH, a blood sample was taken *via* the aorta into a heparinized syringe and the liver was removed quickly.

Analytical Methods——BPB in Plasma: After a blood specimen was centrifuged for 30 min at 3000 rpm, 1 ml of 1 N HCl and 6 ml of iso-amylalcohol were added to 2 ml of the resulting supernatant and the mixture was shaken for 15 min and was centrifuged for 15 min at 3000 rpm. A 5 ml of pH 10 carbonate buffer solution was added to the 3 ml of the resulting iso-amylalcohol phase and the mixture was centrifuged for 15 min at 3000 rpm after shaken for 15 min. The optical density of the resulting water phase was measured at 600 mμ.

PAAH in Plasma: A 1 ml of trichloroacetic acid was added to the mixture of 2 ml of plasma that was obtained by centrifuging a blood sample for 30 min at 3000 rpm and 4 ml of distilled water to precipitate proteins and the resulting mixture was centrifuged for 30 min at 3000 rpm after shaking. A 1 ml of 1 N HCl solution was added to 4 ml of the resulting clear supernatant and the mixture was heated for 45 min at boiling temperature. After cooling, a 4 ml of iso-amylalcohol was added and the resulting mixture was shaken for 30 min and was centrifuged for 10 min at 3000 rpm. A 3 ml of the water phase was taken and was colored by diazo-coupling method with Tsuda reagent (2-diethylaminoethyl-1-naphthylamine). The optical density was measured at 550 mμ.

BPB in Liver: Liver was homogenized in twice its weight of pH 7.4 phosphate buffer solution. After a 6 ml of acetone was added to the 5 ml of the homogenate to extract BPB from liver tissure, the mixture was shaken for 15 min and was centrifuged for 20 min at 3000 rpm. A 6 ml of the resulting supernatant fluid was centrifuged again for 10 min at 3000 rpm to make clear. A 0.1 ml of chloroform was added to 4 ml of the resulting supernatant to dissolve lipids and the optical density was measured at 600 mμ.

PAAH in Liver: A 3 ml of trichloroacetic acid was added to 10 ml of 33% liver homogenate and the resulting mixture was centrifuged for 30 min at 3000 rpm after shaking. A 4 ml of the resulting supernatant was used for hydrolysis and colorled reaction as in the case of plasma.

BPB in Bile: The dye content of a bile sample was determined by diluting the sample with pH 7.4 phosphate buffer solution to a suitable volume and reading the optical density at 600 mμ.

PAAH in Bile: After a bile sample (0.15—0.25 ml) was diluted to 1 ml with distilled water, 5 ml of 1 N HCl solution and 6 ml of iso-amylalcohol were added and the resulting mixture was shaken for 10 min and was centrifuged for 15 min at 3000 rpm. A 5 ml of the resulting water phase was used for hydrolysis and colored reaction as in the case of plasma.

The above analytical methods were developed by the authors and were confirmed to be reproducible.

Liver Cell Suspensions——Rat liver cell suspensions were prepared according to the method of Ichihara, *et al.*,[11] with calcium-free Locke's solution containing 0.027 M sodium citrate. These liver cell suspensions were incubated in a special incubation flask described by Burg and Orloff[12] being bubbled with oxygen gas (95% O_2, 5% CO_2). Being maintained at 37±0.5°, incubation was performed as follows: liver parenchymal cells, 0.5 g, were put into incubation flask and were suspended in 1 ml of Locke's solution. Then a 20 ml of BPB and/or PAAH solution was added to the flask and immediately the mixture was vigorously stirred by continuous supply of O_2. A 3 ml of the suspensions was pipetted off at different time and the cells were separated from the medium by centrifugation for 3 min at 3000 rpm. In the case of BPB, the optical density of the resulting supernatant was measured at 600 mμ. And in the case of PAAH, the resulting supernatant was colored by diazo-coupling method described before and the optical density was measured at 550 mμ. The uptake by the liver cell suspensions was so rapid that these results were expressed as cell/medium concentration ratios at equilibrium state.

Metabolic Inhibitors: Four metabolic inhibitors, dinitrophenol (DNP), KCN, ouabain and iodoacetamide (IAA) were used. A 30 μg/ml of BPB solution was used. BPB solutions containing DNP (0.001 M),

11) Y. Takeda, A. Ichihara, H. Tanioka, and H. Inoue, *J. Biol. Chem.*, **239**, 3590 (1964).
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KCM (0.01 M), ouabain (0.001 M) and IAA (0.01 M) were added to the liver parenchymal cells and the resulting mixture was incubated as above. Analytical method was the same as mentioned above.

Effect of Albumin: Using BPB solution containing bovine serum albumin of which the final concentration was 0.5%, BPB uptake by the liver cell suspensions was studied.

Binding to the Rat Plasma and Liver Homogenate——With equilibrium dialysis cells of which the two chambers were separated by a Visking tube membrane and were equal volumes, the binding of BPB and/or PAAH to the rat plasma was studied. And the binding to the rat liver homogenate was studied according to the method of O'Reilly, *et al.*[13] Shaken overnight at 4°, analysis was performed as mentioned above. And percentage binding to 100% homogenate was determined using 5, 10, 20, and 40% homogenates according to the extrapolation method of Schanker, *et. al.*[14]

Result

Hepato-biliary Transport Characteristics of BPB and PAAH

BPB (mol. wt.=670.02) and BSP (mol. wt.=838.05) contains one and two sulfonic acid groups respectively, but PAAH that is a metabolite of *p*-aminohippuric acid contains one carboxylic acid group and its molecular weight is only 236.12 as shown in Fig. 1.

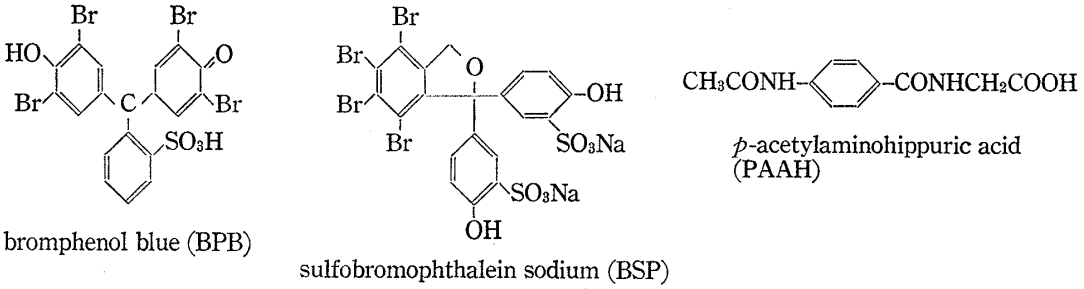

Fig. 1. Chemical Structures of the Organic Anionic Compounds used

These compounds are thought to share the common organic anion transport system of the liver. Though PAAH has been confirmed to be metabolic stable in rat,[8,9,15] the stability of BPB needs to be reconfirmed in rat instead of dog. In experiments in this laboratory, the metabolic stability of BPB has been investigated in bile of the rat. Animals were injected intravenously with BPB (5 mg/300 g body weight) and bile was collected for three consecutive 30 min periods. Paper chromatographic analysis was performed by the three different solvent systems as described by Hart, *et al.*[16] Chromatograms showed one spot and the same *Rf* value as authentic BPB. Accordingly, with respect to both compounds, metabolic process can be excluded. So, these compounds are extremely available for model compounds of the study of hepato-biliary transport.

Table I. Hepato-biliary Transport Characteristic of BPB

Plasma level[a] (μg/ml)	Liver level[a] (μg/g w.wt.)	Bile level[b] (μg/ml)	B/P ratio[c]	L/P ratio[d]	B/L ratio[e]	% excreted in bile for 30 min
3.6±0.6	80.0±8.1	3372±718	894±99	22±5	42±8	55.2±6.5

a) Blood and liver were removed at 30 min after BPB, 5 μmole/300 g body weight, was administered to rats with ligated renal pedicles and the BPB contents were measured. Liver level of BPB is expressed per gram liver wet weight.
b) Bile was collected for three 10-min periods after the administration of BPB and this bile level represents the third period.
c) bile/plasma concentration ratio *d*) liver/plasma concentration ratio *e*) bile/liver concentration ratio
Each value is the mean ± S.E. for 4—6 animals.

13) B.G. Priestly and W.J. O'Reilly, *J. Pharm. Pharmacol.*, **18**, 41 (1966).
14) P.L. Gigon, P.K. Nayak, and L.S. Schanker, *Proc. Soc. Exptl. Biol. Med.*, **132**, 1103 (1969).
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In Table I, the hepato-biliary transport characteristic of BPB is listed. When 5 μmole/300 g body weight of BPB was injected from rat femoral vein, 55.2±6.5% of the injected dose was excreted into bile for 30 min and bile/plasma concentration ratio (B/P ratio) was 894±99. The liver/plasma concentration ratio (L/P ratio) and bile/liver concentration ratio (B/L ratio) were greater than one. These results suggest the possibility that BPB is actively transported from blood into bile.

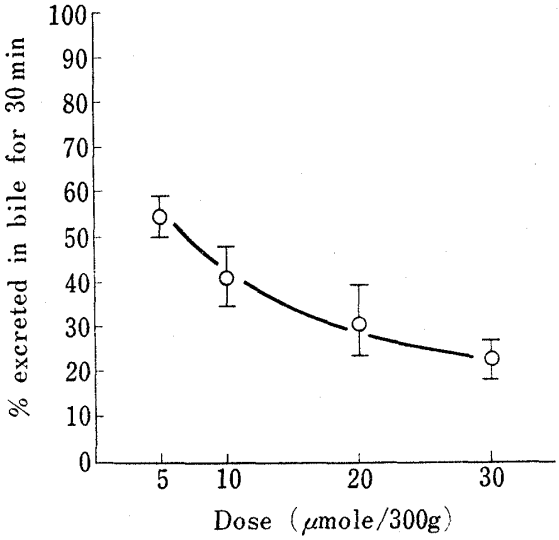

Fig. 2. Dose-dependency of the Hepato-biliary Transport of BPB

After BPB, 5, 10, 20 and 30 μmole/300 g body weight, was administered from rat femoral vein, bile was collected for three 10 min periods and percentage excreted into bile for 30 min was measured.
Each value is the mean±S.E. for 4—6 animals.

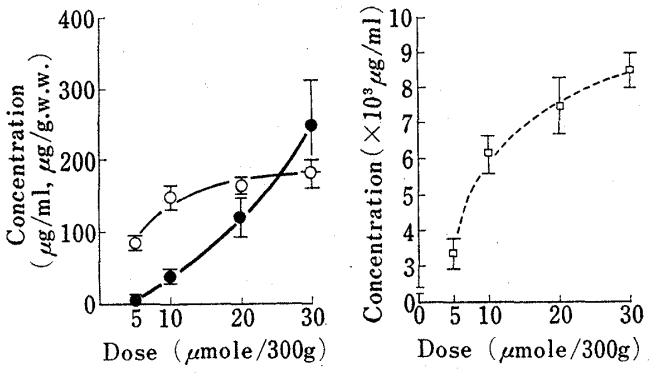

Fig. 3. Dose-dependency of the Plasma, Liver and Bile Levels of BPB

At 30 min after BPB was administered from rat femoral vein, blood and liver were removed and the BPB contents were measured. Bile was collected for three 10 min periods and the BPB content of the third period was measured.
Each value is the mean ± S.E. for 4—6 animals.

●——●: plasma level (μg/ml)
○——○: liver level (μg/g liver wet weight)
□- - - -□: bile level (μg/ml)

Dose-dependency of the hepato-biliary transport of BPB was next studied. In Fig. 2, the relationship between the biliary excretion ratio for 30 min and the injected dose of BPB is demonstrated. When the dose of BPB was increased from 5 to 30 μmole/300 g body weight, the percentage excreted for 30 min was decreased above one-half, from 56% to 22%. In Fig. 3, plasma level (μg/ml), liver level (μg/g liver wet weight) and bile level (μg/ml) at 30 min after several doses of BPB were administered are plotted against doses. As bile flows were 0.02±0.005 ml per 1 min on the average, correctly speaking, bile level is an average from 20 min to 30 min after administration for the sake of analysis. It is thought that the fluctuation of the bile flow was not due to the action of BPB or PAAH but mainly due to the experimental technique. From this figure, it appears that liver level becomes saturated as the dose of BPB is increased. To demonstrate which process, the uptake by the liver from blood and the secretion from the liver into bile, became saturated, L/P ratio and B/L ratio were plotted against injected dose (Fig. 4). When 5 μmole/300 g body weight of BPB was injected from rat femoral vein, L/P ratio was 22±5, but L/P ratio was decreased as the injected dose was increased. On the other hand, B/L ratio was kept the same value, about 43, in these dose ranges. From this observation it is suggested that only uptake process becomes saturated at higher doses and secretory process might have a large capacity.

Effect of BSP on the Hepato-biliary Transport of BPB

BSP (12.5 and 25.0 μmole/300 g body weight) 2 min prior to the intravenous administration of BPB (5 μmole/300 g body weight) was administered and the effect of BSP on the hepato-biliary transport of BPB was examined. At2.5: 1 molar ratio of these compounds, the percentage of total dose of BPB excreted for 30 min was decreased (Table II). At 5: 1

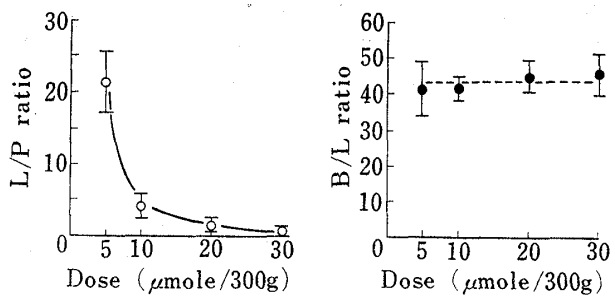

Fig. 4. Dose-dependency of the Liver/Plasma Ratio and Bile/Liver Ratio of BPB

Using the plasma, liver and bile level data of BPB, liver/plasma ratio and bile/liver ratio are plotted against administered dose.
Each value is the mean ± S.E. for 4—6 animals.

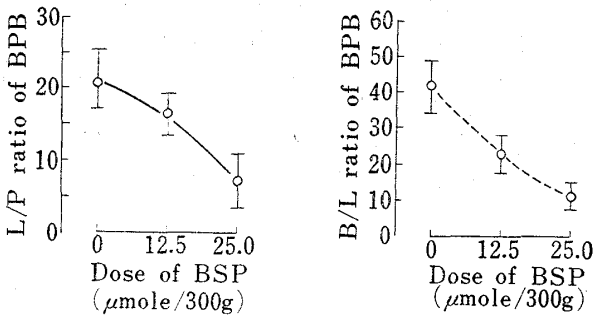

Fig. 5. Effect of BSP on the Hepato-biliary Transport of BPB represented as Liver/Plasma Ratio and Bile/Liver Ratio of BPB

Using the results in Table II, liver/plasma ratio and bile/liver ratio of BPB, 5 μmole/300 g body weight, are plotted against dose of BSP.
Each value is the mean ± S.E. for 4—6 animals.

Table II. Effect of BSP on the Hepato-biliary Transport of BPB

Condition	Plasma level[a] (μg/ml)	Liver level[a] (μg/g w.wt.)	Bile level[b] (μg/ml)	% excreted in bile for 30 min
BSP 12.5 μmole BPB 5.0 μmole	4.5±1.7	90.0±15.0	2010±153	34.9±6.4
BSP 25.0 μmole BPB 5.0 μmole	12.6±1.9	102.2±17.9	1318±152	23.2±3.9

a) After BPB (5.0 μmole/300 g body weight) following BSP (12.5 and 25.0 μmole/300 g body weight) was administered intravenously to rats with ligated renal pedicles, blood and liver were removed at 30 min and BPB contents were measured.
b) Bile was collected for three 10 min periods and these bile levels represent the third periods.
Each value is the mean ± S.E. for 4—6 animals.

molar ratio, the percentage was significantly decreased about 60%, from 55.2 to 23.2. Though bile level was extremely decreased by BSP, liver level was significantly increased.

In Fig. 5, these results are expressed as L/P ratio and B/L ratio. L/P ratio as well as B/L ratio was decreased as the dose of BSP was increased. At 5: 1 molar ratio, both L/P ratio and B/L ratio were reduced about 60%. These results suggest that the two processes, the hepatic uptake and the biliary secretion of BPB are inhibited by the pre-administration of BSP.

Effect of BPB on the Hepato-biliary Transport of PAAH

When PAAH (6 μmole/300 g body weight) was administered from rat femoral vein, the percentage of the total dose excreted for 30 min was 21.4±3.0% and its B/P ratio was much greater than one, but L/P ratio was less than one. These results suggest that PAAH is actively transported from plasma into bile as BPB (Table III). When BPB (15 and 30 μmole/300 g body weight), 2 min prior to the intravenous administration of PAAH (6 μmole/300 g body weight), was administered to rat intravenously, the percentage of the total dose of PAAH excreted for 30 min was significantly decreased and liver level as well as bile level was decreased (Table IV). In Fig. 6, plotting L/P and B/L ratios of PAAH against the dose of BPB, it becomes clear that both L/P ratio and B/L ratio of PAAH were significantly decreased by BPB (level of significance=5%).

Uptake by the Liver Cell Suspensions

To study uptake process from blood to the liver, the liver cell suspensions were used *in vitro*. The uptake rate of BPB from the medium to the liver cells was so rapid that the result was expressed as cell/medium concentration ratio (C/M ratio) at an equilibrium state.

Table III. Hepato-biliary Transport Characteristic of PAAH

Plasma level[a] (μg/ml)	Liver level[a] (μg/g w.wt.)	Bile level[b] (μg/ml)	B/P ratio	L/P ratio	B/L ratio	% excreted in bile for 30 min
11.4±3.5	5.4±1.6	570±15	43±15	0.47±0.27	120±30	21.4±3.0

a) At 30 min after PAAH, 6 μmole/300 g body weight, was administered intravenously to rats with ligated renal pedicles, blood and liver were removed and PAAH contents were measured.
b) Bile was collected for three 10 min periods after the administration of PAAH and this bile level represents the third period.
Each value is the mean ± S.E. for 4—6 animals.

Table IV. Effect of BPB on the Hepato-biliary Transport of PAAH

Condition	Plasma level[a] (μg/ml)	Liver level[a] (μg/g w.wt.)	Bile level[b] (μg/ml)	% excreted in bile for 30 min
BPB 15 μmole PAAH 6 μmole	26.2±3.3	3.7±0.7	135±30	5.0±1.0
BPB 30 μmole PAAH 6 μmole	22.6±3.0	3.9±0.5	72.2±8.8	3.3±0.8

a) After PAAH (6 μmole/300 g body weight) following BPB (15 and 30 μmole/300 g body weight) was administered intravenously to rats with ligated renal pedicles, blood and liver were removed at 15 min and PAAH contents were measured.
b) Bile was collected for three 10 min periods and this bile level represents the third period.
Each value is the mean ± S.E. for 4—6 animals.

In Fig. 7, a relationship between C/M ratio and the initial concentration of BPB in the medium is demonstrated. As the initial concentration of BPB in the medium was increased, the C/M ratio was decreased. So, it appears that the capacity of the BPB uptake from the medium to the liver cells becomes limited as the initial concentration is increased. With this liver cell suspensions, the effect of BSP on the uptake of BPB was studied. BSP inhibited the uptake of BPB by the liver cells as shown in Fig. 8, similar to the L/P ratio *in vivo* in Fig. 5. Next, the effects of several metabolic inhibitors on the uptake of BPB were studied. When DNP (0.001 M), KCN (0.01 M), ouabain (0.001 M) and IAA (0.01 M) were added to the medium, the C/M ratios of BPB became 22.2, 21.6, 18.6 and 18.3 respectively. So, these metabolic inhibitors do not inhibit the uptake of BPB by the liver cell suspensions. When bovine serum albumin was added to the medium of which the albumin concentration was 0.5%, the C/M ratio of BPB became significantly decreased (C/M ratio=0.8).

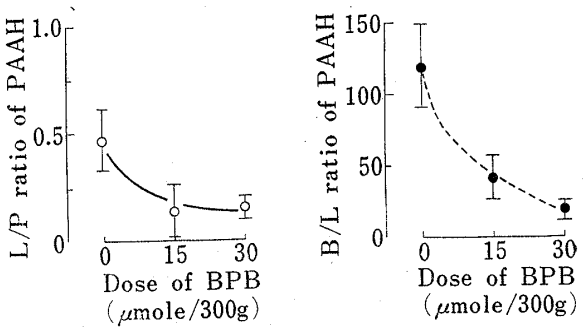

Fig. 6. Effect of BPB on the Hepato-biliary Transport of PAAH represented as Liver/Plasma Ratio and Bile/Liver Ratio of PAAH

Using the results (Table III and IV), liver/plasma ratio and bile/liver ratio of PAAH, 6 μmole/300 g body weight, are plotted against does of BPB.
Each value is the mean±S.E. for 4—6 animals.

Binding of BPB and PAAH to the Rat Liver Homogenate and Plasma

It was suggested that protein binding in the liver must be important in the hepatic uptake of some compounds.[13,14] To test this possibility, the ability of the rat liver homogenate and plasma to bind BPB and PAAH was examined (Table V). Though the degrees of the binding of BPB to rat plasma and the liver homogenate were extremely high (95.9 and 95.6%), the degree of the binding of PAAH to the liver homogenate was low (11.3%). It appears that the great difference of the L/P ratio between BPB and PAAH is mainly due to the degree of the binding to the liver homogenate.

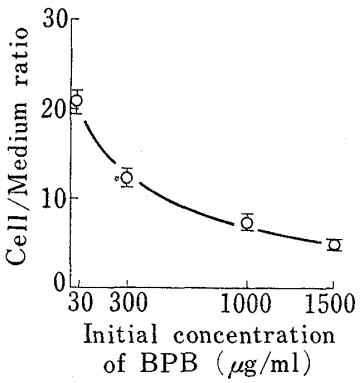

Fig. 7. Dose-dependency of the Uptake of BPB by Liver Cell Suspensions

To the liver parenchymal cells (0.5 g), 20 ml of BPB solution (30, 300, 1000 and 1500 μg/ml) and 1 ml of Locke's solution were added and the mixture was incubated at 37°. After 3 min, a sample was removed from medium and BPB content was measured. The uptake of BPB by the liver cell suspensions is expressed as medium/cell concentration ratio.

Each value is the mean±S.E. for 4—6 animals.

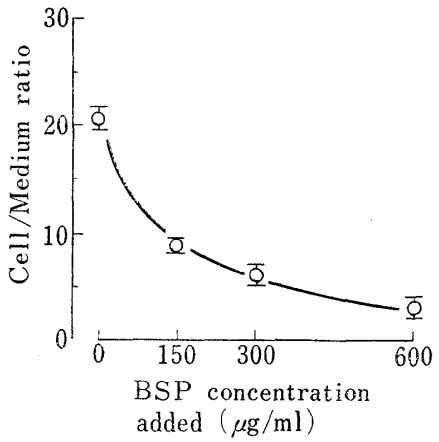

Fig. 8. Effect of BSP on the Uptake of BPB by the Liver Cell Suspension

To the liver parenchymal cells (0.5 g), 10 ml of BSP solution (150, 300 and 600 μg/ml) and the equal volume of BPB solution (30 μg/ml) and 1 ml of Locke's solution were added and the resulting mixture was incubated. After 3 min, a sample was removed from medium and the BPB content was measured.

Each value is the mean±S.E. for 4—6 animals.

Table V. Binding of BPB and PAAH to the Rat Plasma and Liver Homogenate

Compounds	% bound to plasma[a)]	% bound to liver homogenate[b)]
BPB	95.9± 1.7[c)]	95.6±1.5[d)]
PAAH	58.8±11.4[e)]	11.3±3.4[f)]

a) Using cells for equilibrium dialysis, binding to the rat plasma was examined. The cells having two chambers of which the volumes were equal were shaken during two days at 4°.
b) Binding to the rat liver homogenate was examined according to the methods of Schanker *et al.* (ref. 16) and percentage binding was measured by extrapolation of 5, 10, 20 and 40% homogenates.
c) Total concentration (free plus bound) range of BPB was from 250 to 847 μg/ml.
d) Total concentration range of BPB was 250—2100 μg/g homogenate.
e) Total concentration range of PAAH was 47—181 μg/ml.
f) Total concentration range of PAAH was 46—400 μg/g homogenate.

Discussion

As the assay method for the liver level of BPB or PAAH used in this report is to extract them from the whole liver tissues, this method, which is similar to the method of Klaassen,[17] measures the amount of these compounds not only in the liver cells but also in blood which is trapped in the liver and in bile canaliculi and ductules. By Whelan, *et al.*,[6] the amount of the drug which was trapped in hepatic blood was corrected but Schanker said in his report[18] that no correction factors were applied to the hepatic values of compounds since these would not significantly affect the major conclusions. Thus with respect to liver levels, no correction is applied in this report. From our results, it is thought that BPB and PAAH are actively transported into rat bile because their excretion percentages into bile for 30 min were dependent on the administered dose and the B/P ratios were extremely greater than one and the inhibitory phenomena between BPB and BSP and between BPB and PAAH were observed.

As the liver levels of these model compounds were measured in this study, the two steps namely the uptake by the liver from blood and the secretion from the liver into bile can be

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discussed. Brauer[19] originally divided the substances which were excreted into bile into three classes according to their B/P ratios: Substances of class A are those whose ratio is nearly one (glucose, Na^+, Ka^+, and Cl^-), class B include substances whose B/P ratios usually range from 10 to 1000 (bile salts, BSP, fluorescein, rose bengal *etc.*) and class C compounds consist of those in which the ratio is less than one (inulin, sucrose, phosphate and mucoproteins). According to his classification, both BPB and PAAH belong to class B compounds. Though with respect to B/P ratio, BPB and PAAH appear to have the same hepato-biliary transport characteristics, the state of PAAH in the liver is thought to completely differ from that of BPB because of the difference of their L/P ratios; L/P ratio$>$1 for BPB and L/P ratio$<$1 for PAAH. From this point of view, liver level must be measured in the study of biliary excretion, though liver level was not often measured. Taking account of liver level, organic anions that are actively excreted into bile had better to be classified into two groups:

i) bile level$>$liver level$>$plasma level (BPB type)
ii) bile level$>$plasma level$>$liver level (PAAH type)

This distinction is thought to be due to the uptake by the liver parenchymal cells. Though using rat liver slices many studies have been done about hepatic accumulation,[20,21] this is not a suitable method for hepatic uptake process, because plasma membranes of the liver parenchymal cells may be often broken. Accordingly using the liver cell suspensions, the hepatic uptake was studied in this report. The facts that the uptake of BPB by the liver cell suspensions became saturated as the initial dose of BPB was increased and that BSP inhibited the uptake of BPB suggest the possibility that active transport type process, facilitated diffusion[22] or pinocytosis,[23,24] and protein binding process participate in the hepatic uptake of these compounds. But the fact that various metabolic inhibitors did not influence the uptake of BPB by the liver cell suspensions denies the idea of active transport process. On the other hand, as both BPB and PAAH had affinities to the liver homogenate, it is thought that the uptake process by the liver cells from blood is due to the binding to the liver homogenate. And with respect to this point, detailed investgation will be done in our next report.

About secretory process, few studies have been done because of its complexity and of difficulty in research technique. Though about the biliary excretion of lipophilic compounds such as imipramine and its metabolite desmethylimipramine[25] and phosphatidylcholine,[26,27] micelle formation has a important function, the micellar interaction is not probable in the case of BPB and PAAH because of their hydrophilicity. But generally speaking, active transport is thought to take part in this process. Our results that B/L ratio of BPB was kept nearly an infinite value, about 43, in the dose range used in this study and that B/L ratio of PAAH (6 μmole/300 g body weight) was 120±30 support this hypothesis and it is thought that BPB, BSP and PAAH are secreted in bile by the same active transport system because of significantly depressed B/L ratio of BPB by BSP and of extremely depressed B/L ratio of PAAH by BPB. About the fact that the liver level of BPB was increased about 10—20% by the pre-administration of BSP, our next report will explain by means of introducing the function of the liver cytoplasmic binding protein "Ligandin."[28]

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